H.K. Dermatol. Venereol. Bull. (2003) **11**, 161-168

Reports on Scientific Meeting

Annual Scientific Meeting of The Hong Kong Society of Dermatology & Venereology 2003

Reported by PT Chan 陳寶德, W Chan 陳弘, YH Chan 陳耀海

Date: 29 June 2003
Venue: Sheraton Hotel, Hong Kong
Organizers: The Hong Kong Society of Dermatology and Venereology

The use of recombinant-antigen enzyme immunoassay as a serological screening test for syphilis in people with human immunodeficiency virus infection

Speaker: Dr. K. K. Ho
Medical Officer, Social Hygiene Service, Department of Health

Syphilis has a number of clinical manifestations including a period of asymptomatic infection and so serological screening tests are crucial for detection and control of the disease. However, it is common for patients infected with Human Immunodeficiency Virus (HIV) to have unusual serological responses, such as false positivity seen with traditional treponemal antigen tests (FTA-Ab, VDRL, TPHA), seronegative secondary syphilis, seroreversion and slow decline of titre after treatment. A new recombinant antigen-based enzyme immunoassay (EIA) (ICE, Murex Diagnostics) has been reported to have a higher sensitivity than other treponemal antigen tests. Dr. Ho carried out a cross sectional study to assess the prevalence of syphilis and the performance characteristics of FTA-Abs and ICE Syphilis EIA as a serological screening test in Kowloon Bay Integrated Treatment Centre.

Four hundred and forty-eight eligible patients were interviewed and 433 (83.6% male, 16.4% female) completed the twelve-week study in September to December 2001. There were 48 patients with diagnosis of syphilis (prevalence 11.1%). Among them, 8.3% were primary syphilis, 12.5% were secondary syphilis, 14.6% were early latent syphilis, 60.4% were late latent syphilis and 4.2% were neurosyphilis. There were 30 inconclusive results in FTA-Abs and none in the ICE Syphilis EIA. The sensitivity and false negative predictive values for the two tests were comparable in the study. The total number of false positive results was lower in ICE Syphilis EIA compared to FTA-Abs.

Due to the lack of control, the results cannot be generalised to HIV seronegative subjects from this study. Only eight patients were newly diagnosed to have syphilis during the study period and the other had a past history with adequate treatment. The cost and applicability of the ICE syphilis EIA test were not addressed in the study.

Learning points:

The commonest presentation of syphilis among HIV patients was late latent syphilis. ICE syphilis EIA in this group of patients was sensitive, specific with high negative and positive predictive values and low inconclusive results. This make ICE syphilis EIA a reliable screening tool in HIV patients in our local setting.

Mycosis fungoides – a study of forty cases in Hong Kong

Speaker: Dr. L. S. Ku
Medical Officer, Social Hygiene Service, Department of Health

Mycosis fungoides (MF) is the commonest primary cutaneous T-cell lymphoma. In year 2001, the speaker performed a multi-clinic, 35-year retrospective study to determine the clinico-pathological characteristics, treatment and outcomes of MF/Sezary Syndrome (SS) patients in Social Hygiene Service (SHS). All skin biopsy records and subsequently their case records (if applicable) from 1967 to July 2001 in all major SHS clinics were reviewed. Eventually 39 cases of MF and one SS were studied.

The male to female ratio was 2.1:1 and mean age at diagnosis was 56.4 years. There were on average 1.17 MF cases per 10,000 new skin cases per year in SHS from 1988 to 2000 and the estimated incidence of MF in Hong Kong (HK) was 0.44/100,000. The incidence in Hong Kong in this study was much lower than reported in the West and the mean age at diagnosis was younger, though the HK incidence in this study was likely to be an underestimation (even SHS is taking care of the majority of dermatology patients). Six patients had history of occupational exposure to petrochemical products and radioactive substances. The average duration from symptoms onset to diagnosis was 95.6 months. It took a mean of 1.48 skin biopsies and an average of 12.7 months to establish the diagnosis. Atypical lymphocytes were seen in 91.9% of the biopsies.

Fifty-five percent of the patients were staged with the TNM system. Among them 77.3% were Stage I, 4.5% Stage IIa, 4.5% Stage IIb, 9% Stage IVa and 4.5% Stage IVb. Twenty-eight patients were treated with PUVA and 18 (78.3%) achieved complete remission. Average maintenance PUVA therapy was 9.5 months only. The relapse rate was 66.7% and the mean duration of remission was 19 months. The high relapse rate and shorter remission compared to the literature raised the issue of the need of a prolonged course of PUVA. Five patients received total skin electron beam therapy and the rate of complete remission was 40% and relapse rate was 100%. Progression rate in early stage was 9.9% but 42% in late stage. The overall all 5-year survival was 88% but 100% for Stage I.

Learning points:

A high index of suspicion with earlier skin biopsies may lead to early diagnosis of MF. A more prolonged course of PUVA is indicated to achieve better clinical outcomes. A local registry of MF will be useful for standardisation of diagnosis, staging and treatment of MF and the causal role of occupation can be further verified.

Oncogenic human papillomavirus infection – epidemiology and associated risk factors in local high-risk women

Speaker: Dr. W. K. Tang
Medical Officer, Social Hygiene Service, Department of Health

Certain human papillomavirus (HPV) types such as 16, 18, 31, 33, 52 and 58 possess malignant potential. The prevalence of these oncogenic HPV in patients with high-risk sexual behaviour or human immunodeficiency virus (HIV) has not been reported locally. The speaker carried out a cross sectional study in a HIV centre (Kowloon Bay Integrated Treatment Centre, Special Preventive Program, Department of Health) and two sexually transmitted diseases clinics (Yau Ma Tei and Yung Fung Shee Social Hygiene Clinics). All female attendees who fulfilled the inclusion criteria between March and September 2001 were recruited after consent.

Cervical smears were collected and interpreted by liquid-based method (ThinPrep) and tested for HPV by a combination of type specific polymerase chain reaction (PCR) for types 16, 18 and 58, and nucleotide sequencing techniques for the nested PCR products, followed by comparison of the sequences with the GenBank database for determination of the HPV type.

Two hundred and forty-five females were recruited with median age of 35. Forty women were HIV-1 positive. One hundred and seventeen women (48.3%) were diagnosed HPV positive (total 29 types different HPV were identified). Fifty-eight women (24%) had oncogenic HPV and types 16, 18, 52 were the commonest ones. Three patients had multiple infections. Among patients with oncogenic HPV, 33 (56.9%) had subclinical oncogenic HPV infection and 11 (19%) were co-infected with HIV-1. No low risk HPV was detected in HIV-1 positive patients. One hundred and ninety women (49% of total) had normal cervical cytology. Thirty-eight participants (15.5%) had cytological abnormalities including atypical squamous cells of undetermined significance (ASCUS) (4.9%), low-grade squamous intraepithelial lesion (9.8%) and high-grade squamous intraepithelial lesion (HSIL) (0.8%). There was no invasive cancer. Most (56.9%) of the oncogenic HPV detected were from subjects of normal cytology. But the speaker reminded us that all HSIL cases in this study were positive for oncogenic HPV. The prevalence of oncogenic HPV in the study group was 24% but higher among HIV positive women.

Learning points:

HIV-1 positivity confers a higher risk for oncogenic HPV infection. HPV testing is potentially useful to triage HIV-1 patients with ASCUS for early colposcopic investigation. Education, behavioural modification and regular Paps smear are important in the prevention of cervical malignancy.

Surgical management of cutaneous tumours

Speaker: Dr E. C. K. Chan
Specialist in Plastic Surgery, Department of Surgery, Division of Plastic and Reconstructive Surgery, Prince of Wales Hospital

Skin is the largest organ in the body and cutaneous tumours are the commonest tumours encountered by clinicians. Eighty-six percent of basal cell carcinoma (BCC) and 66% of squamous cell carcinoma (SCC) are located in the head and neck region and there are enormous numbers of cutaneous tumours that are to be removed due to cosmetic and personal reasons.

A skin biopsy is required to confirm or disprove a clinical diagnosis. It should be made at the most developed and untreated area and common methods include shave, punch, incisional and excisional biopsies.

The extent of surgical excision of a skin tumour depends on the size, shape, site, location and whether it is primary or recurrent. For primary BCC, a 4 mm margin gives 98% clearance (tumours <2 cm in diameter) but for recurrent BCC, 5 to 10 mm margin gives a five-year cure rate of 83%. The deep margin should include the superficial or mid-subcutaneous fat.

For well differentiated SCC at low risk sites, not involving the fat, a 4 mm margin is adequate but for a poorly differentiated SCC with diameter less than 2 cm at low risk site or more than 1 cm at high risk sites, a 6 mm margin is adequate. The deep margin should include the subcutaneous layer.

For melanoma in-situ or malignant melanoma with thickness less than 1 mm, a 2.5 mm margin will result in a five-year survival of 95 to 100%. For tumours between 1 to 2 mm, a 1 to 2 cm margin gives a five-year survival rate of 80 to 96%. A margin of 2 to 3 cm is required for tumours between 2 to 4 mm in order to achieve a five-

year survival rate of 60 to 75% and for tumours more than 4 mm in diameter, a margin of 2 to 3 cm is required to achieve 50% five-year survival. Deep margin should be down to but not including the muscle fascia and for lesions on the scalp, it should be down to the subgaleal plane.

Undermining is a technique for reducing closure tension. It should be done at the level of subdermal fat on the face, at the level of subcutaneous fat on the trunk and at the level of subgaleal layer on the scalp. Closures with sutures at the dermal and subcutaneous layers also reduce closure tension for better cosmetic results and facilitate earlier suture removal.

Skin grafts can be divided into full thickness grafts and partial thickness grafts. Partial thickness grafts have better success take rate which is important in large area grafting, whilst full thickness grafts have better pigmentation match, textural match and graft function giving an overall better aesthetic outcome.

A flap is a tissue structure that contains its own intra-vascular circulation and is transferred (versus a graft which depends on circulation of the recipient site). A flap can be divided into random pattern flap (with blood supply from the edges) and axial pattern flap (with its own anatomically identifiable blood supply). Axial pattern flaps include cutaneous, fascio-cutaneous and musculo-cutaneous flaps. A local flap is a flap that obtained from an area near the lesion with similar texture and skin colour. Examples include: rotation flaps after removal of facial skin tumours especially those over the side of scalp where a skin graft will result in a completely hairless area. Axial pattern flap has its own blood supply and can survive even if it bears cartilage or bone. Large BCC on the nose may be excised followed by reconstruction with a naso-labial flap on a cartilage graft from ear. Similarly, radiation induced sarcoma on chest wall can be repaired by a Gortex graft covered by a distant flap with subcutaneous fat. A free flap is a skin flap obtained from one part of the body such as the arm and is then transferred to somewhere else. After removal of the free flap, the arm survives with the ulnar artery and ulnar arch.

In patients with large congenital melanocytic naevus on the scalp, a tissue expander is sometimes used before surgery to ensure sufficient tissue remains after excision for wound closure.

Skin graft cannot be used to repair a tissue defect after tumour removal on weight bearing areas of the sole as continuous friction causes graft breakdown. The Y-to-V flap can repair such difficult cases using skin from the insole with its own blood supply.

Learning points:

Plastic surgery plays an important role in the diagnosis and treatment of skin tumours. With the use of various special techniques such as undermining, tissue expander and axial pattern skin flap, better aesthetic and functional outcomes can be achieved.

Activating smoothened mutations in sporadic basal cell carcinoma

Speaker: Dr. C. W. Lam
Associate Professor, Department of Chemical Pathology, Prince of Wales Hospital

Sporadic basal cell carcinomas (BCCs) are the commonest form of skin cancer in fair-skinned adults with over a million cases a year worldwide. However, the molecular mechanisms underlying the development of sporadic BCCs remained unknown.

Basal cell nevus syndrome (BCNS) is an autosomal dominant disease characterised by the presence of multiple BCCs, odontogenic keratocysts,

palmoplantar pits, calcification in the falx cerebri and associated with other carcinomas such as childhood medulloblastoma. It is caused by mutational inactivation of the PATCHED gene (PTCH). PTCH is the human homologue of the Drosophila segment polarity gene, patched (Ptc), which encodes a putative twelve-span transmembrane receptor protein for the secreted molecule, Sonic hedgehog (Shh). In the absence of Shh signal, Ptc represses the constitutive signalling activity of the smoothened gene (SMO), by forming a Ptc.SMO complex. Mutational inactivation of PTCH results in the failure of PTCH to inhibit SMO, leading to constitutive activity of the Shh signalling pathway.

To investigate the molecular basis of BCNS in Chinese, the speaker performed mutational analyses of PTCH gene in four BCNS families by a sensitive mutation detection method using denaturing high performance liquid chromatography (DHPLC). Results show that mutational inactivation of the PTCH gene causes BCNS in Chinese. Two insertion and one deletion mutations affecting coding sequence and one deletion mutation affecting a splicing site were identified. All the mutations cause a shift in the open reading frames and lead to premature termination of PTCH protein translation.

DHPLC can be completed in six minutes. By mixing the patient's DNA with the wild type DNA, heteroduplex and homoduplex DNA can be obtained. In the presence of mutation, more than two peaks will be seen. This can help to identify the exon bearing the mutation. Nucleotide sequencing can then be performed using the automated DNA sequencer to delineate the mutation.

The human SMO gene is composed of twelve exons within 24 kb of genomic DNA and has been mapped to chromosome 7q31-32 by in-situ hybridisation. It codes for a protein that consists of 787 amino acids and is a seven-span transmembrane protein.

Two SMO gene mutations have been found in sporadic BCCs. One, at base pair 1604 (G-to-T transversion) of exon 9 (numbering according to Genbank accession number U84401) and this mutation changes codon 535 from tryptophan to leucine, whilst the other, at base pair 1685 (G-to-A transition) of exon 10 and such mutation changes codon 562 from arginine to glutamine.

The evidence that these mutations cause cancer can be provided by DNA transfection in mouse. If mutated SMO gene is transfected, transformation will occur which result in tumour formation whilst mouse transfected with wild type SMO gene will not have transformation.

In order to determine the frequency and genotype-phenotype correlation of the two SMO mutations in our sporadic BCCs, these two mutations were screened by restriction analysis and direct sequencing.

A total of 97 formalin-fixed paraffin-embedded BCCs (72 nodular, 16 infiltrative, 4 micronodular, 5 superficial spreading) from 97 patients were obtained from the Department of Anatomical and Cellular Pathology, Prince of Wales Hospital; Department of Pathology, Princess Margaret Hospital and Department of Pathology, United Christian Hospital. Polymerase chain reaction (PCR) was performed with SMO gene primers to amplify the target DNA sequence. For exon 9, the PCR products of the wild-type allele contain two BstN1 restriction sites while the 1604G→T transversion abolishes one site and results in an extra fragment of size 42 bp larger than that from the wild-type. For exon 10, the mutation 1685 G→A transition, abolishes the only AlwI restriction site in the PCR products. Direct sequencing of the PCR products was performed using a terminator cycle sequencing kit and an automated DNA sequencer The 1604G→T mutation, identified by BstN1 restriction analysis, was detected in 20 out of 97 (20.6%) sporadic BCCs. Fourteen out of a total of 72 nodular (19.4%), Five out of a total of 16 infiltrative (31.3%), and one out of a total of

five superficial spreading (20%) whilst the 1685 G→A mutation, identified by AlwI restriction analysis, was not detected in any of the BCCs, indicating that this SMO mutation is not a common one in the samples.

In conclusion, the high prevalence of the 1604 G→T transversion in the speaker's samples indicates that this mutation is important in the carcinogenesis of BCCs in Hong Kong, and may be a mutational hot spot for the human SMO gene.

Another objective of this study was to determine whether SMO is also involved in the carcinogenesis of medulloblastomas. Fresh frozen tissues from 21 sporadic medulloblastomas from 21 patients were obtained from the Department of Anatomical and Cellular Pathology, Prince of Wales Hospital and these medulloblastoma samples were screened for two SMO mutations by restriction analysis. Finally, the 1604G→T mutation was found in one out of 21 medulloblastomas and this sample come from a three-year-old boy with classical midline cerebellar medulloblastoma, desmoplastic variant. In conclusions, the finding of the 1604G→T mutation in medulloblastomas suggests that the sonic hedgehog-patched-smoothened signalling pathway also plays an important role in the carcinogenesis of this tumour. In view of these findings, some pharmaceutical companies in the United States are trying to develop blockers that can block this pathway in order to shrink the tumour and eliminate the need for surgical intervention.

Learning points:

With the use of restriction analysis and nucleotide sequencing, activated smoothened mutations are found to play an important role in the pathogenesis of sporadic basal cell carcinoma and medulloblastoma.

Diagnosis and treatment of primary cutaneous lymphoma in Chinese

Speaker: Dr. W. Y. Au
Associate Consultant, Department of Medicine, Queen Mary Hospital

Skin is the largest organ in the body. Lymphocytes and antigen presenting cells are an integral part of skin defense mechanism. T-lymphocytes in the skin are not static as they recirculate into the lymphatic and blood stream. Homing to skin of T-lymphocytes is possible through receptors such as cutaneous lymphocyte antigen. Primary cutaneous lymphoma accounts for 4.1% of all lymphoma cases in the Queen Mary Hospital.

Most of the primary cutaneous lymphomas are T or NK cell lymphomas. The reverse is true for systemic lymphomas in which B cell lymphomas are more common. Lymphomas are different from reactive lymphoid infiltrates in being clonal, proliferative and destructive if left untreated. Clonality can be assessed by T cell receptor polymerase chain reaction studies. Surface antigens on lymphocytes can be used for classification of lineage such as CD20 in B cell lymphomas and loss of CD7 in mycosis fungoides. The diagnosis of malignant lymphoma is also suggested by an abnormal kappa to lambda ratio in B cell lymphomas or an abnormal CD4/CD8 ratio in T cell lymphomas. The classification of lymphomas is heterogeneous and is ever evolving. The World Health Organization classification is developed in the perspective of oncologists and pathologists whereas the system of the European Organization for Research and Treatment of Cancer is developed in the perspective of dermatologists.

Mycosis fungoides is derived from CD4+ helper/memory epidermotropic T cells. Early stage disease responds to local treatment like steroids, retinoids and phototherapy. As the disease progresses, electron beam therapy may be used. The survival benefit of new agents such as bexarotene and denileukin diftitox remains to be established.

Primary cutaneous CD 30-T cell lymphoma usually presents as cutaneous nodules. It responds favourably to chemotherapy with a five-year survival of above 90%. Lymphomatoid papulosis presents with recurrent and self-limiting papules. Some cases of lymphomatoid papulosis can progress to CD30-T cell lymphomas or other lymphomas (including Hodgkin and B cell lymphoma).

Nasal-type NK/T cell lymphoma primarily occurs in the oriental population. The nose is preferentially affected and skin is the next common site. Necrotic angioinvasive lesions are common presenting features. Expression of Epstein Barr Virus (EBV) encoded small nuclear RNA (EBER) is often present and circulating EBV DNA can be used for monitoring disease progress. The prognosis of disseminated disease is poor and bone marrow transplant may be tried.

Subcutaneous panniculitic T cell lymphoma is rare. It presents with subcutaneous inflammatory nodules and systemic symptoms. Fludarabine has been used successfully but the fat atrophy can be permanent.

B cell cutaneous lymphoma is rare. Light chain restriction and demonstration of clonal IgH rearrangement confirms disease. Primary cutaneous B cell lymphoma is rare as compared to that of T cell counterpart. Careful staging procedures are necessary to rule out systemic involvement.

New therapies for lymphomas are discussed. The first one is antibody directed to the surface antigens of lymphoma cells. A number of antibodies directed to different surface antigens of lymphoma cells can be used. There is no cross toxicity and the effect is synergistic. Antibody can be used alone to mediate antibody dependent cellular cytotoxicity, or it can be tagged with toxin or radioactive isotope in mediating tumour cell killing. Differentiating agents like bexarotene (retinoic acid X receptor retinoid) and arsenic trioxide has also been used. Bexarotene has been approved for treatment of cutaneous T cell lymphoma. Nucleoside analog such as fludarabine is another treatment option. It has been used successfully in subcutaneous panniculitic T cell lymphoma. Antisense obligonucleotides are in development and they mediate cellular killing through their effects on signal transduction. Immunotherapies like bone marrow transplant or tumour vaccine are also potential options in lymphoma treatment.

Learning points:

Most primary cutaneous lymphomas belong to T cell lineage. Careful staging examinations are required for newly diagnosed cutaneous B cell lymphomas to rule out systemic involvement.

The role of radiotherapy in the management of skin cancer

Speaker: Dr. W. M. Sze
Senior Medical Officer, Department of Clinical Oncology, Pamela Youde Nethersole Eastern Hospital

Skin cancer has a lower incidence in our population as compared with the western countries. In Hong Kong, it accounted for 2.6% of all new cancer cases and 0.4% of cancer deaths in 1999. There are different types of skin cancers arising from various structures of skin. Radiotherapy acts by inducing deoxyribonucleic acid damage. Different types of skin cancers have their own susceptibility to radiotherapy, with lymphomas being most sensitive and melanoma and sarcoma being less radiosensitive. Common primary skin cancers such as basal cell carcinoma (BCC) and squamous cell carcinoma have intermediate radiosensitivity. For radiosensitive tumours, radiotherapy is an effective local therapy whereas for less radiosensitive tumours, radiotherapy can be used as an adjuvant or for palliative purposes.

The advantages of radiotherapy include its lack of risk of anaesthesia, more liberal margin, no need for reconstruction and less dependence on the operators. But it requires more clinic visit for treatment and carries certain late side effects such as occurrence of skin atrophy, telangiectasia, necrosis of skin or adjacent structures and risk of secondary malignancy. Moreover, margin control is difficult for highly invasive tumours with indistinct borders. Choice of treatment for skin tumours depends on availability of expertise and patient factors. Elderly patients with high surgical risk and cancers in the head and neck region, which require extensive reconstruction, are common candidates for radiotherapy. Choice of radiotherapy should also be dependent on radiosensitivity of the selected tumour types. Even in less radiosensitive tumours, radiotherapy can be used as adjuvant or palliation of pain, bleeding or bulk reduction.

Basal cell carcinoma (BCC) is the commonest primary skin cancer. It rarely metastasized and can be cured by local treatment. Typical tumour control rate after primary radiotherapy varies from 90% to 95%. There are two randomised control trials on radiotherapy of BCC. The first one compared cryotherapy with radiotherapy. Although the cosmetic results were similar for both treatments, cryotherapy had a higher four-year relapse rate of 39% as compared with four percent in the group treated with radiotherapy. The second trial compared radiotherapy with surgery. Surgery had a lower relapse rate compared with radiotherapy (0.7% versus 7.5%). In addition, cosmetic results were also better with surgery. However, 93% of patients in this trial had tumour <2 cm and 91% of surgery had margins controlled by frozen section. Therefore the result may not be totally applicable to large tumours or surgery without meticulous margin control.

Mycosis fungoides is a primary T-cell lymphoma. It is more radiosensitive as compared with BCC and the main tumour burden is located in the skin (except when systemically involved in the late stage). Radiotherapy therefore plays a role in its management. It can be given as total skin electron beam therapy (TSEBT), which results in 95% complete remission rate in stage IA disease. About half of stage IA patients remained in remission at five years. Although high complete remission rate can be achieved in higher stage diseases, most patients will relapse in the long run. Side effects of TSEBT include skin atrophy, telangiectasia, alopecia, hypohidrosis and subcutaneous fibrosis. Radiotherapy can also be used as adjuvant to vegetative lesions or as palliation in mycosis fungoides.

Learning points:

Radiotherapy is useful for management for skin cancers and good collaboration between dermatologists and radiotherapists is essential for optimal treatment results.